Phenylethylamine as a Biochemical Marker of Tiger

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Pheromones of the tiger happen to be a mixture of amines which are "fixed" by lipids. One of these, found always in all the four tigers investigated, is phenylethylamine. This may be a biochemical marker at a species level.

Pheromones, although well known in the insect world, have attracted scanty attention in mammals. The present status of pheromone research on vertebrates has been summed up in a recent symposium [1].

We now report the chemical analysis of pheromones of tigers. A characteristic musky odour of tigers is a well known phenomenon. In his first hand study of wild tigers Schaller [2] observed the squirting of this musky fluid on three occasions.

As far as we could establish on the basis of our observation of Khairi, a tigress raised up in Jashipur, Orissa, India from early cubhood in 1974 to the present state of maturity, this smell is contained in a white, lipid-rich fluid which is squirted through the urinary channel. Schaller [2] mentioned the difference in the angle of squirting by tigers and tigresses.

We have been collecting samples of this marking fluid and/or urine of Khairi since June 1976. We have also compared samples obtained from two other tigresses and a tiger in the zoo.

Steam distillation of the samples separates the smell from lipids and other components. The smelly distillate is highly alkaline and ninhydrin positive. The material was rendered acidic with the help of HCl and spotted for chromatography.

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Thin layer chromatography and paper chromatography were carried out with the solvent system n-butanol : aceticacid : water(4 : 1 : 1). In all the samples from Khairi collected in 1976 and 1977 there were two ninhydrin positive spots, apart from ammonia *(i. e.* hydrochloride form). From September 1978 onwards, there appeared more ninhydrin spots (five to six). In one series of experiments a longitudinal strip of the chromatographic paper was cut out and stained with ninhydrin. By laying it along the remaining wider strip, the positions of the spots on the latter were traced with pencil. (This procedure was also adopted for eluting). On moistening with alkali these pieces of paper emitted an odour which was perceptible to seven unbiased persons. Strong ammoniacal smell issued from the ammonia spot.

In all the four tigers studied, one amine, the fastest migrating, was always prominently visible whereas comparison of two adult animals, one male and one female, revealed a difference in some of the amines as evident from R_f value and colour.

Assuming that the pheromonal message communicates information at the species and individual level, this "tigeramine" with the high R_f value (which is absent in the ordinary cat and golden cat [3], a rare Indian species) is likely to be a biochemical marker at the species level.

We therefore tried to determine its chemical nature. Electrophoretic run on Whatman paper with acidic and alkaline buffer (buffers: Tris 7.5 and phosphate buffer 6.5, 200 V, 5–7 mA) with standard monoamine and diamine showed that the tigeramine moves with the monoamine. Mass spectrograph showed a molecular ion of molecular weight 121. Fragments 30 and 91 suggest CH_2NH_2 and $CH_2C_6H_5$. These facts indicate that the tigeramine is phenylethylamine. Standard β-phenylethylamine (Sigma) was used as a reference in a chromatographic run with Khairi's sample and the tigeramine and β-phenylethylamine were seen to have the same R_f value.

The smell of the marking fluid of tiger is perceptible to the human nose for days and weeks but the distillate (which elicited the characteristic "flehmen" response of Khairi) rapidly evaporates and loses the odour. It is thus likely that the lipids act as a "fixative" for the smell in order to linger it for many days. This is of great ecological and ethological significance, for it enables a passing animal to decode the message concerning the presence of a conspecific

for many days. The distillate or pure phenylethylamine can be artificially "fixed" with vaseline etc.

It also seems reasonable that the pattern of amines in the marking fluid changes with age.

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[1] D. Müller-Schwarz and M. M. Mozell, (eds), Chemical signals in vertebrates, Plenum Press, 1977.
[2] G. Schaller, The Deer and the Tiger, Chicago University Press, 1967.
[3] R. L. Brahmachary and S. Chattopadhyaya, Science and Culture (Calcutta). **44**, 258 (1978).